

#4



PATENT
Docket No.: 19603/3356 (CRF D-1595F)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s) :	Barany et al.)	Examiner:
Serial No. :	09/963,920)	Unknown
Cnfrm. No. :	1149)	
Filed :	September 26, 2001)	Art Unit:
For :	DETECTION OF NUCLEIC ACID SEQUENCE)	2857
	DIFFERENCES USING THE LIGASE)	
	DETECTION REACTION WITH)	
	ADDRESSABLE ARRAYS)	

COPY OF PAPERS
ORIGINALLY FILED

SUPPLEMENTAL PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231
Box: Non-Fee Amendment

Dear Sir:

Please amend the above-identified patent application as follows:

In the Specification:

Please substitute the pending paragraphs at page 9, lines 30-31, page 9, lines 32-33, page 43, line 30 to page 44, line 10, and page 45, line 21 to page 46, line 2 with amended paragraphs at page 9, lines 30-31, page 9, lines 32-33, page 43, line 30 to page 44, line 10, and page 45, line 21 to page 46, line 2 as follows:

At page 9, lines 30-31:

Figures 21A-F show a schematic cross-sectional view of the synthesis of an addressable array, in accordance with Figures 19B-C.

At page 9, lines 32-33:

Figures 22A-C are schematic views of an apparatus used to synthesize the 8 x 8 array of 24 mers on a solid support in accordance with Figures 19B-C, 20A-C, and 21A-F.

At page 43, line 30 to page 44, line 10:

The starting surfaces will contain free amino groups, a non-cleavable amide linkage will connect the C-terminus of PNA to the support, and orthogonal side-chain deprotection must be carried out upon completion of segment condensation assembly in a way that PNA chains are retained at their addresses. A simple masking device has been designed that contains 200µm spaces and 200µm barriers, to allow each of 5 tetramers to couple to the solid support in distinct rows (Figure 20A). After addition of the first set of tetramers, the masking device is rotated 90°, and a second set of 5 tetramers are added (Figure 20B). This can be compared to putting icing on a cake as rows, followed by icing as columns. The intersections between the rows and columns will contain more icing, likewise, each intersection will contain an octamer of unique sequence. Repeating this procedure for a total of 6 cycles generates 25 squares containing unique 24-mers, and the remaining squares containing common 12-mers (Figures 20C and 21A-F). The silicon or glass surface will contain 10µm ridges to assure a tight seal, and chambers will be filled under vacuum. A circular manifold (Figure 26) will allow for circular permutation of the six tetramers prior to delivery into the five rows (or columns). This design generates unique 24-mers which always differ from each other by at least 3 tetramers, even though some sequences contain the same 3 tetramers in a contiguous sequence. This masking device is conceptually similar to the masking technique disclosed in Southern, et al., Genomics, 13:1008-1017 (1992) and Maskos, et al., Nucleic Acids Res., 21:2267-2268 (1993), which are hereby incorporated by reference, with the exception that the array is built with tetramers as opposed to monomers.

At page 45, line 21 to page 46, line 2:

Figures 21A-F show a schematic cross-sectional view of the synthesis of an addressable array (legend). Figure 21A shows attachment of a flexible spacer (linker) to surface of array. Figure 21B shows the synthesis of the first rows of oligonucleotide tetramers. Only the first row, containing tetramer 1, is visible. A multi-chamber device is placed so that additional rows, each containing a different tetramer, are behind the first row. Figure 21C shows the synthesis of the first columns of oligonucleotide tetramers. The multi-chamber device or surface has been rotated 90°. Tetramers 9, 18, 7, and 12 were added in adjacent chambers. Figure 21D shows the second round synthesis of the oligonucleotide rows. The first row contains tetramer 2. Figure 21E shows the second round of synthesis of oligonucleotides. Tetramers 34, 11, 14, and 23 are added in adjacent chambers during the second round. Figure 21F shows the structure of the array after third round synthesis of columns (the first row contains tetramer 3), adding tetramers 16, 7, 20, 29. Note that all 24-mer oligonucleotides within a given row or column are unique, hence achieving the desired addressable array. Since each 24-mer differs from its neighbor by three tetramers, and tetramers differ from each other by at least 2 bases, then each 24-mer differs from the next by

1875	1876	1877	1878	1879	1880	1881	1882	1883	1884	1885	1886	1887	1888	1889	1890	1891	1892	1893	1894	1895	1896	1897	1898	1899	1900	1901	1902	1903	1904	1905	1906	1907	1908	1909	1910	1911	1912	1913	1914	1915	1916	1917	1918	1919	1920	1921	1922	1923	1924	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936	1937	1938	1939	1940	1941	1942	1943	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	1962	1963	1964	1965	1966	1967	1968	1969	1970	1971	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100	2101	2102	2103	2104	2105	2106	2107	2108	2109	2110	2111	2112	2113	2114	2115	2116	2117	2118	2119	2120	2121	2122	2123	2124	2125	2126	2127	2128	2129	2130	2131	2132	2133	2134	2135	2136	2137	2138	2139	2140	2141	2142	2143	2144	2145	2146	2147	2148	2149	2150	2151	2152	2153	2154	2155	2156	2157	2158	2159	2160	2161	2162	2163	2164	2165	2166	2167	2168	2169	2170	2171	2172	2173	2174	2175	2176	2177	2178	2179	2180	2181	2182	2183	2184	2185	2186	2187	2188	2189	2190	2191	2192	2193	2194	2195	2196	2197	2198	2199	2200	2201	2202	2203	2204	2205	2206	2207	2208	2209	2210	2211	2212	2213	2214	2215	2216	2217	2218	2219	2220	2221	2222	2223	2224	2225	2226	2227	2228	2229	2230	2231	2232	2233	2234	2235	2236	2237	2238	2239	2240	2241	2242	2243	2244	2245	2246	2247	2248	2249	2250	2251	2252	2253	2254	2255	2256	2257	2258	2259	2260	2261	2262	2263	2264	2265	2266	2267	2268	2269	2270	2271	2272	2273	2274	2275	2276	2277	2278	2279	2280	2281	2282	2283</
------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	--------

REMARKS

Pursuant to 37 CFR § 1.121, attached as Appendix A is a Version With Markings to Show Changes Made.

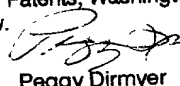
In view of the all of the foregoing, applicants submit that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

Date: December 20, 2001

Georgia Evans
Georgia Evans
Registration No. 44,597

NIXON PEABODY LLP
Clinton Square, P.O. Box 31051
Rochester, New York 14603
Telephone: (716) 263-1672
Facsimile: (716) 263-1600

Certificate of Mailing - 37 CFR 1.8(a)	
I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, Washington, D.C. 20231, on the date below.	
Date 12/20/01	 Peggy Dirmyer

Appendix A

Version With Markings to Show Changes Made

In reference to the amendments made herein to the specification, additions appear as double-underlined text, while deletions appear as bracketed text, as indicated below:

In the Specification:

At page 9, lines 30-31:

Figures [21A-G] 21A-F show a schematic cross-sectional view of the synthesis of an addressable array, in accordance with Figures 19B-C.

At page 9, lines 32-33:

Figures 22A-C are schematic views of an apparatus used to synthesize the 8 x 8 array of 24 mers on a solid support in accordance with Figures 19B-C, 20A-C, and [21A-G] 21A-F.

At page 43, line 30 to page 44, line 10:

The starting surfaces will contain free amino groups, a non-cleavable amide linkage will connect the C-terminus of PNA to the support, and orthogonal side-chain deprotection must be carried out upon completion of segment condensation assembly in a way that PNA chains are retained at their addresses. A simple masking device has been designed that contains 200µm spaces and 200µm barriers, to allow each of 5 tetramers to couple to the solid support in distinct rows (Figure 20A). After addition of the first set of tetramers, the masking device is rotated 90°, and a second set of 5 tetramers are added (Figure 20B). This can be compared to putting icing on a cake as rows, followed by icing as columns. The intersections between the rows and columns will contain more icing, likewise, each intersection will contain an octamer of unique sequence. Repeating this procedure for a total of 6 cycles generates 25 squares containing unique 24-mers, and the remaining squares containing common 12-mers (Figures 20C and [21A-G] 21A-F). The silicon or glass surface will contain 10µm ridges to assure a tight seal, and chambers will be filled under vacuum. A circular manifold (Figure 26) will allow for circular permutation of the six tetramers prior to delivery into the five rows (or columns). This design generates unique 24-mers which always differ from each other by at least 3 tetramers, even though some sequences contain the same 3 tetramers in a contiguous sequence. This masking device is conceptually similar to the

masking technique disclosed in Southern, et al., Genomics, 13:1008-1017 (1992) and Maskos, et al., Nucleic Acids Res., 21:2267-2268 (1993), which are hereby incorporated by reference, with the exception that the array is built with tetramers as opposed to monomers.

At page 45, line 21 to page 46, line 2:

Figures [21A-G] 21A-F show a schematic cross-sectional view of the synthesis of an addressable array (legend). Figure 21A shows attachment of a flexible spacer (linker) to surface of array. Figure 21B shows the synthesis of the first rows of oligonucleotide tetramers. Only the first row, containing tetramer 1, is visible. A multi-chamber device is placed so that additional rows, each containing a different tetramer, are behind the first row. Figure 21C shows the synthesis of the first columns of oligonucleotide tetramers. The multi-chamber device or surface has been rotated 90°. Tetramers 9, 18, 7, and 12 were added in adjacent chambers. Figure 21D shows the second round synthesis of the oligonucleotide rows. The first row contains tetramer 2. Figure 21E shows the second round of synthesis of oligonucleotides. Tetramers 34, 11, 14, and 23 are added in adjacent chambers during the second round. Figure 21F shows the structure of the array after third round synthesis of [PNA rows. The first row contains tetramer 3. Figure 21G shows the structure of the array after third round synthesis of] columns[, (the first row contains tetramer 3), adding tetramers 16, 7, 20, 29. Note that all 24-mer oligonucleotides within a given row or column are unique, hence achieving the desired addressable array. Since each 24-mer differs from its neighbor by three tetramers, and tetramers differ from each other by at least 2 bases, then each 24-mer differs from the next by at least 6 bases. Each mismatch significantly lowers T_m , and the presence of 6 mismatches in just 24 bases would make cross hybridization unlikely even at 35°C. Note that the smaller 12-mer sequences are identical with one another, but are not at all common with the 24-mer sequences. Even though the particular 12-mer sequence may be found within a 24-mer elsewhere on the grid, for example 17-1-2-3-28-5, an oligonucleotide will not hybridize to the 12-mer at temperatures above 50°C.